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Seedlings growth performance of *Azadirachta indica* A. Juss. seeds collected from avenue plantation

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Abstract
The discharges of different types of pollutants due to automobile exhaust emission are widely believed to have detrimental effects on plant growth. The effects of automobile exhaust emission on the seedling growth performance of *Azadirachta indica* A. Juss avenue tree was observed. Poor germination and seedling establishment are major problems in arid and semi-arid environments, and these characteristics are considered to be important factors in later plant growth and yield especially for plants growing in the polluted environment. The seeds germination behavior of *A. indica* seeds collected from both polluted (Avenue planting) and less polluted environment (fields) was investigated. Growth performance of polluted and less polluted area seeds showed a great variation in different growth variables in natural field conditions. Root length and leaf area of polluted environment seeds were significantly ($p<0.05$) reduced as compared to the seeds of the less polluted areas. Shoot length, seedling size, number of leaves, circumference and seedling dry biomass were significantly ($p<0.05$) declined in polluted area seeds when compared with the seeds of less polluted areas. Moreover, root/shoot ratio and specific leaf area were promoted from the seeds of polluted areas. Leaf weight ratio was significantly low in the seeds of polluted areas. The seeds of the less polluted environment significantly maintained leaf area ratio in comparison with the seedlings grown from the seeds of the polluted areas. Tolerance test showed that seedling growth of *A. indica* showed lowest percentage of tolerance in seeds collected from Shahrah-e-Faisal (63.61%) followed by University road (71.09%), North Nazimabad (78.15%) and Shaheed-e-Millat road (98.31%) as compared to University Campus.
Key words: Avenue plantation, *Azadirachta indica* seeds, Karachi, polluted areas, seedling growth

Introduction

Karachi is the Pakistan's largest industrial and commercial center, handling much of Pakistan's international trade. It is situated at 24^0 51'36"N 67^0 0'36"E on the shore of Arabian Sea near the Indus River delta. As one of the most rapidly growing cities in the world, Karachi faces challenges that are central to many developing metropolises, including traffic congestion and environmental pollution. Traffic problems and pollution are major challenges for Karachi. The rapid increase in the number of motor vehicles in the city in recent years has resulted in a high degree of traffic pollution that has been identified as one of the most important factors contributing to deterioration of the city's environmental quality. The uncontrolled growth in urbanization and motorization generally contributes to an urban land use and transportation system that is environmentally unsustainable [1]. Air pollution is a major part of over all atmospheric pollution and motor vehicle usually constitute the most significant source of ultra fine particles in an urban environment [2]. The inhibitory effects in leaf pigments concentration, nitrate reductase activity and cambial activities of *Azadirachta indica* (Neem tree) due to pollution stress was observed [3].

Transport vehicles greatly pollute the environment through emissions such as CO, CO_2, NO_x, SO_x, unburnt or partially burnt HC and particulate emissions. Fossil fuels are the chief contributors to urban air pollution and major source of green house gases (GHGs) and considered to be the prime cause behind the global climate change [4]. SO_2, which is a major component of air pollutants, affects morphological characteristics of plants such as number of leaves, leaf area, length of stem and roots and number of flowers and fruits [5]. Total chlorophyll, carotenoid, leaf area, plant height, fresh, dry biomass, corm yield, corm number, corm size, flower production, length of stigma+style, fresh and dry biomass of flower of *Crocus sativus* L. were significantly reduced by automobile emission [6]. The flowering in plants growing at polluted site is delayed and there was a marked reduction in flowering density, flowering period, and size of floral parts, pollen fertility, fruit and seed set. These changes were found to be closely associated with the extent of air pollution caused mainly by significant in the number of automobiles [7]. Traffic pollution has resulted in significant accumulation of heavy metals in both the roadside leaves and soils [8].

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Azadirachta indica is a flowering plant, belongs to family meliaceeae and economically an important commonly known as neem tree. The parts of this tree have been used for medicine, shade, building materials, fuel and lubrication. Sultana [9] reported that the growth of *Aspergillus parasiticus* and alfatoxins production were inhibited during storage of three important cereals (wheat, maize and rice) using leaves of neem (*Azadirachta indica*). Traditionally and commercial products are derived from neem to control disease and for pest management (*Azadirachta indica* A. Juss) leaves in Pakistan [10].

The aim of the present study was to investigate the impact of automobile pollution on seeds of *A. indica* at different roads of Karachi and growth performance of seedlings polluted and less polluted seeds of *A. indica* growing in natural field conditions.

Materials and Methods

The healthy seeds of *Azadirachta indica* A. Juss. were collected randomly from the University Campus, University Road, Board Office Road, Shaheed-e-Millat Road and Shahrah-e-Faisal (Fig. 1).

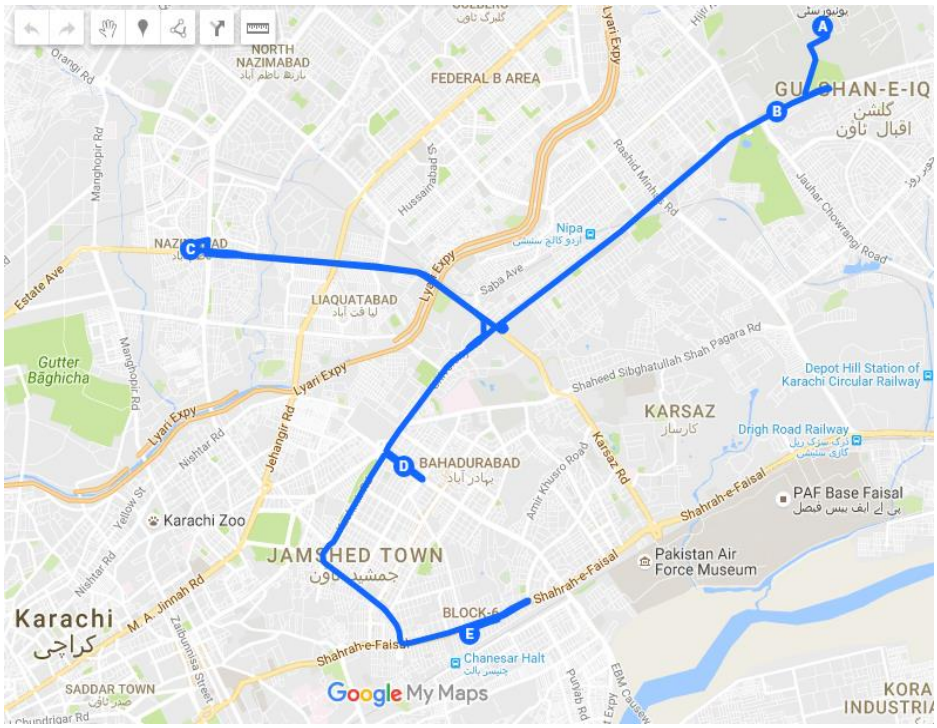

Fig. 1. Study sites map. Symbol used: A= the University Campus, B =University Road, C =Board Office Road, D=Shaheed-e-Millat Road, E= Shahrah-e-Faisal.
Ack. Source: Study sites accessed with the help of Google My Maps.

The collected seeds were germinated in natural environmental conditions at the Department of Botany, University of Karachi. The micropyler ends of seeds were slightly cut with a clean scissor to remove any possible seed coat dormancy. The seeds were sown in large pots having garden soil. Only one seedling was transplanted after 10 days of germination in each small pot of 20 cm in diameter and 9.8 cm in depth having garden soil at 1 cm depth and watered regularly. There were five replicates for each treatment. Pots were reshuffled weekly to avoid light/ shade or any other climatic effects. Seedling height, number of leaves, leaf length and plant cover (plant circumference) was noted. After eight weeks, seedlings were taken out from pots and washed their roots with tape water. Data on seedling root and shoot length, root-shoot ratio and seedling fresh and dry weight was obtained. Seedling dry weight was determined by drying the plant materials in an oven at 80°C for 24 hours. Root/shoot ratio, leaf weight ratio, specific leaf area and leaf area ratio were calculated by the formulae as mentioned by Rehman and Iqbal [11]. Traffic density was recorded for 6-hours/day (2-

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hours in the morning, afternoon and evening, respectively) at University Campus, University road, North Nazimabad (Board Office), Shaheed-e-Millat road and Shahrah-e-Faisal to investigate the traffic load in peak hours and their effects on *A. indica*. Tolerance indices were determined by the following formula:

Tolerance Indices = Mean root length of seedling of polluted area seeds/Mean root length of seedling of campus seeds X 100

Data analysis

The obtained data was statistically analyzed by Analysis of Variance (ANOVA) and Duncan's Multiple Range Test at $p < 0.05$ level on personal computer using SPSS-13 software.

Results

The seedlings emerged from the seeds collected from University Campus showed better growth as compared to polluted and less polluted roads of the Karachi city. Traffic density was recorded high at Shahrah-e-Faisal followed by University road, North Nazimabad (Board Office) and Shaheed-e-Millat road, while in University Campus traffic density was much low (Fig. 2).

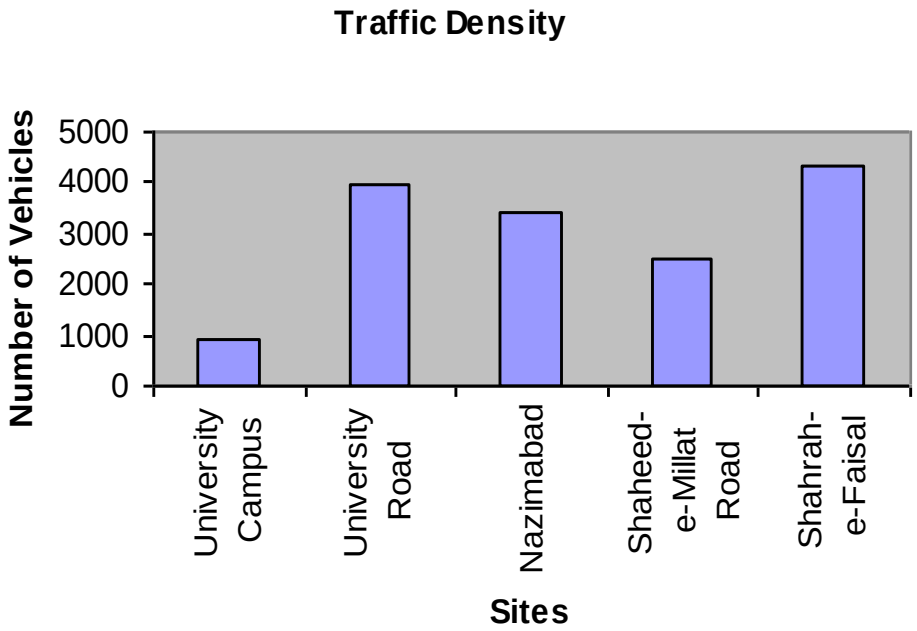

Fig. 2: Traffic density/hour at different roads of Karachi in May, 2009.

Root length was significantly ($p<0.05$) affected and recorded as 8.46, 9.30 and 7.57cm for the seeds collected from the University Road, Board Office and Shahrah-e-Faisal, respectively as compared to University Campus (11.90cm) and Shaheed-e-Millat Road (11.70cm). Shoot length significantly ($p<0.05$) declined with 14.25, 15.73, 17.53 and 12.63cm of the seedlings sprouting from the seeds collected form University Road, Board Office, Shaheed-e-Millat Road and Shahrah-e-Faisal, respectively when compared to the shoot length (22.43cm) of University Campus seedlings. Seedling size, number of leaves, circumference and seedling dry biomass were also significantly decreased of the seedlings developing from the seeds collected from the University Road, Board Office, Shaheed-e-Millat Road and Shahrah-e-Faisal as compared to University Campus seeds (Table 1). Leaf area was recorded 3.01, 3.18cm^2 for the seedlings of seeds collected from University Campus and Shaheed-e-Millat Road, respectively, while this was significantly low 1.62, 2.19 and 1.23cm^2 as recorded from the seeds collected from University Road, Board Office and Shahrah-e-Faisal, respectively. Root/shoot ratio represented 0.41 and 0.39 high values for University Campus and Shahrah-e-Faisal as compared to University Campus, Board Office and Shaheed-e-Millat road which showed low

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values as 0.29, 0.30 and 0.29, respectively. Leaf weight ratio was significantly reduced and recorded as 0.26, 0.23 and 0.23 for the seeds of University Road, Board Office and Shahrah-e-Faisal respectively, while the seeds of University Campus and Shaheed-e-Millat Road represented comparatively high values 0.31 and 0.31. Specific leaf area was increased 14.38 and 14.50 $cm^2\ g^{-1}$ for the seeds of Board Office and Shahrah-e-Faisal, respectively and the seeds of University Campus, University Road and Shaheed-e-Millat Road showed 10.62, 13.57 and 12.93 $cm^2\ g^{-1}$, respectively. The seedlings emerging from the seeds collected from the University Campus, University Road, Board Office and Shahrah-e-Faisal showed significant ($p<0.05$) reduction in leaf area ratio as 3.30, 3.50, 3.33 and 3.27 $cm^2\ g^{-1}$, respectively as compared to Shaheed-e-Millat Road which showed 4.09 $cm^2\ g^{-1}$.

Tolerance indices were also applied on the seedlings of *A. indica* emerging from the seeds collected from polluted and less polluted areas of Karachi. The seeds collected from Shahrah-e-Faisal showed low percentage of tolerance indices in seedling growth as compared to seeds collected from polluted (North Nazimabad) and less polluted (Shaheed-e-Millat road) areas of Karachi city. The seedlings of *A. indica* showed moderate percentage of tolerance at University road and North Nazimabad (Board Office) road while the seeds collected from Shahed-e-Millat road showed highest tolerance indices (Fig. 3).

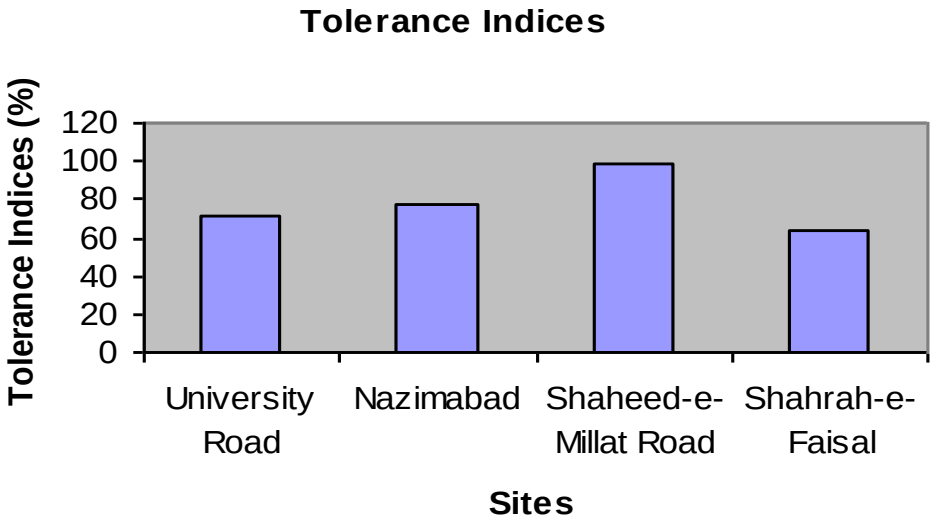

Fig. 3: Tolerance Indices of *Azadirachta indica* plants raised from the seeds collected from different roads of Karachi.

Table 1. Effects of auto emission on growth performance of *Azadirachta indica* seeds collected from different roads of Karachi growing in natural field conditions.

Sites	Root length (cm)	Shoot length (cm)	Seedling size (cm)	No. of leaves	Leaf area (cm^2)	Circumference (cm)	Seedling dry wt. (g)	Root/shoot ratio	Leaf wt. ratio	Specific leaf area ($cm^2\ g^{-1}$)	Leaf area ratio ($cm^2\ g^{-1}$)
A	11.90±0.67a	22.43±0.47a	34.33±0.74a	28±0.88a	3.18±0.12a	36.67±0.88a	0.91±0.05a	0.29±0.01b	0.31±0.05a	10.62±0.48c	3.30±0.11b
B	8.46±0.34bc	14.25±0.34cd	22.73±0.06d	17±0.57c	1.62±0.03c	24.33±1.20c	0.46±0.08d	0.39±0.02a	0.26±0.02b	13.57±0.53b	3.50±0.12ab
C	9.30±0.25b	15.73±0.69c	25.03±0.91c	20±0.88b	2.19±0.17b	29.33±0.89b	0.56±0.05c	0.30±0.05b	0.23±0.03b	14.38±1.38a	3.33±0.27b
D	11.70±0.26a	17.53±0.71b	29.23±0.78b	22±0.57b	3.01±0.14a	32.00±1.15b	0.78±0.08b	0.29±0.01b	0.31±0.08a	12.93±0.69b	4.09±0.19a
E	7.57±0.29c	12.63±0.38d	20.20±0.25e	13±0.87d	1.23±0.04d	20.66±0.88d	0.38±0.01e	0.41±0.03a	0.23±0.02b	14.50±1.81a	3.27±0.23b
	Number followed by the same letters in the same column are not significantly different according to Duncan Multiple Range Test at $p<0.05$ level. ± Standard Error **Symbol used:** **A** = University Campus **B** = University Road **C** = Board Office Road **D** = Shaheed-e-Millat Road **E** = Shahrah-e- Faisal										

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Discussion

The Karachi city is suffering with high environmental pollution due to industrialization and motorization. Germination is considered the first and an important stage in establishment of trees and regeneration of wild species [12]. The traffic density is high and vehicles passing through busy roads produce toxic effects on the plants growing near the roadsides avenue planting. In the present study, the growth of *A. indica* was greatly affected at Shahrah-e-Faiasl and University Road due to high traffic load which was investigated by growing the seeds collected from Shahrah-e-Faisal and University road. Same results were found at Pir Vadhai, one of the busiest and polluted roads of Rawalpindi near Islamabad, having a high traffic load and which was recorded approximately 1100-1200 vehicles/hour at peak hours and produced harmful effects on experimental fields of wheat crop [13]. Pollution caused by traffic activities is becoming a great threat to urban environmental quality, human health and as well as plants. High traffic load contributes greater dustfall on the plant leaves which also depends upon the condition of roads and size and structure of leaves [14]). The seed germination and seedling growth is sensitive to automobile pollution. The seedling growth of *A. indica* was much affected for the seeds collected from Shahrah-e-Faisal and University road, the highly polluted area of the city. The pollutants derived from the autoexhaust emission can directly affect the plant ability to produce and store food in the seeds [15]. The results are in agreement with the findings of Ganatsas [16]. The seeds (reproductive material) of *Pinus brutia* collected at a distance of 0-30 m from close the ring road of Thessaloniki (Greece) were unable to germinate, whereas, seeds of *P. brutia* collected over to 100 m distance from close the ring road showed common germination behavior.

The shoot length, seedling size, number of leaves, circumference and seedling dry biomass of *A. indica* were significantly reduced for seeds collected from the University road, North Nazimabad, Shaheed-e-Millat Road and Shahrah-e-Faisal as compared to the seeds collected from the University Campus. These results were verified with the results of Shafiq and Iqbal [17] that the growth of *Cassia siamea* was significantly affected in seeds collected from M.A. Jinnah road, Shahrah-e-Faisal, Nazimabad and Gulshan-e-Iqbal as compared to University Campus. They have also recorded that root length, seedling length and seedling dry weight were significantly decreased for the seedlings emerged from the seeds collected from polluted areas of the city as compared to University Campus. The reduction in growth variables of *A. indica* in polluted areas of the city might be due to the development of unhealthy seeds produced by tees growing along the main polluted roads of the city. Due to occurrence near the roads, the autoexhaust emissions and particulate matter could be deposited on the leaf surfaces and other parts of plants. The deposition of autoexhaust emissions and particulate matter on the leaf surface caused clogging of stomates and ultimately reduced the photosynthetic rate. The chlorophyll and carotenoid both takes part in photosynthetic reaction. The different pollutants derived from autoexhaust emissions play a significant role in inhibition of photosynthetic activity that may results in depletion of chlorophyll and carotenoid content of the leaves of various plants [18].

The growth performance of *A. indica* seeds collected from the polluted sites of the city like University road, North Nazimabad, Shaheed-e-Millat and Shahrah-e-Faisal was severely distressed as compared to seeds collected from the University Campus which showed better growth performance. The present study was supported by the investigations of Kabir *et al.* [19] who demonstrated that *Samanea saman* growth was badly affected at Shahrah-e-Faisal as compared to University Campus. According to them, phenology, leaf area, leaf fresh and dry weights, pods size, number of seeds per pod, seed length and breadth of *Samanea saman* were affected greatly by auto emission on Shahrah-e-Faisal as compared to other city roads and University Campus. The ecological effects of autoexhaust emissions on plants have been reviewed by many workers [20]. Bernhardt-Romermann *et al*. [21] have reported changes in plant community composition with increasing proximity to roadsides in Germany. Gratani *et al*. [22] establish positive relationships between traffic density and photosynthetic activity, stomatal conductance, total chlorophyll content and leaf senescence of *Quercus ilex* L. Roads and traffic in the urban areas severely disturbed the environment due to which plants growth and physiolological

reactions were inhibited Ahmed *et al.,* [23]. The impact and resistance to petroleum product and under roadside automobile pollution stress on germination, physiological and morphological of plant species were also reported [24-26]. Khan and Abbasi [27] concluded that trees, shrubs and other vegetation can absorb and assimilate certain air pollutants according to their tolerable limits.

Conclusion

It was concluded from the present study that the growth performance of *A. indica* seeds collected from the polluted sites of the city like University road, North Nazimabad, Shaheed-e-Millat and Shahrah-e-Faisal was greatly distressed as compared to seeds collected from the University Campus which showed better growth performance. It was also concluded that the seeds of *A. indica* showed low percentage of tolerance ability at polluted roads of Karachi like Shahrah-e-Faisal and showed high percentage of tolerance at less polluted or in comparatively clean areas.

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